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Acetylcholinesterase biosensor based on single-walled carbon nanotubes—Co phtalocyanine for organophosphorus pesticides detection

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ABSTRACT

A simple and reliable technique has been developed for the construction of an amperometric acetylcholinesterase biosensor based on screen-printed carbon electrodes. For the first time, one-step modification using single-walled carbon nanotubes and Co phtalocyanine has been proposed to decrease the working potential and to increase the signal of thiocholine oxidation. The biosensor developed made it possible to detect 5–50 ppb of paraoxon and 2–50 ppb of malaoxon with detection limits of 3 and 2 ppb, respectively (incubation 15 min). The biosensor showed high reproducibility when measurements of the substrate and inhibitor were performed (R.S.D. about 1% and 2.5%, respectively). The reliability of the inhibition measurements was confirmed by testing spiked samples of sparkling and tape waters.

1. Introduction

Cholinesterase biosensors have attracted considerable attention since 1990-s [1,2] due to the importance of the analytes detected which involve insecticides [3], nerve gases [4], alkaloids [5], fluorides and surfactants [6]. The main attention is focused on organic species which inhibit cholinesterase in ultra-small quantities and hence exert a high acute toxicity toward human beings. The presence of insecticides or nerve gases traces results in the irreversible suppression of cholinesterase activity followed by cessation of the nerve impulse transduction. In cholinesterase biosensors, the decay of enzyme activity is quantified as a measure of the inhibitor content by recording the biosensor signal prior to and after its contact with a sample. Most part of the research is devoted to acetylcholinesterase (AChE) preferably presented in blood serum and considered as a primary target of such agents [7] even though some other enzyme sources have been successfully applied for biosensor assembly [1,2], with recombinant enzymes isolated from mutant microorganisms [8], among them.

Electrochemical, fiber-optic and fluorometric transducers have been employed in cholinesterase sensor assembling. Among them, electrochemical devices became most popular due to inexpensive equipment and simple operation in laboratory and field conditions [1]. The amperometric detection of the AChE activity is mainly based on direct or mediated oxidation of the product of enzymatic hydrolysis of thiocholine ester (1), an artificial analog of acetylcholine, a natural neural transmitter [9].

$$\begin{aligned} &\text{Acetylthiocholine} + H_2O \xrightarrow{\text{ChE}} \text{thiocholine} + \text{acetic acid} \\ &2\,\text{Thiocholine} - 2e^- \rightarrow \text{dithio} - \text{bis} - \text{choline} + 2H^+ \end{aligned} \quad (1)$$

The direct oxidation of thiocholine (1) on bare electrodes requires rather high voltage coupled with the fouling problem of the working electrode surface. For this reason, various mediator systems have been employed, e.g., ferrocyanide [10], Prussian Blue [11], tetracyanoquinodimethane (TCNQ) [8], Co phtalocyanine [12] and carbon nanotubes (CNTs) [13]. The application of mediators significantly decreases the working potential as well as improves reproducibility and sensitivity of thiocholine detection. Most mediators are implemented in the electrode materials (carbon paste or carbon ink of screen-printed electrodes) or placed onto the surface of the working electrode. This limits the enzyme "wiring" because of the little electroactive contact area left. For this reason, CNTs are of special interest due to their ability both to form 3D-nets with high adsorptive activity toward enzymes and to establish electric contact with the electrode. These features of CNTs are most significant for oxidoreductases [14]. However, the CNTs mediation of thiocholine oxidation was also found very promising for the improvement of inhibition detection. In fact, the immobilization of multi-walled CNTs and AChE in polyelectrolyte multilayers made it possible to decrease the working potential to +150 mV and reach a high sensitivity of detection of model anticholinesterases [13]. The physical adsorption of AChE onto multi-walled CNTs allowed recording signal of thiocholine oxidation at +200 mV and mea-

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